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Galactomannan Antigenemia after Infusion of Gluconate-Containing Plasma-Lyte▽

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We demonstrated that sodium gluconate was the factor causing false-positive galactomannan (GM) antigenemia of Plasma-Lyte hydration solution. Infusion of sodium gluconate-containing solution but not gluconate-free Plasma-Lyte resulted in positive serum GM antigenemia. Serum GM concentrations also correlated with the volume and *in vitro* concentrations of GM within gluconate-containing solutions of infused Plasma-Lyte.

Galactomannan (GM) antigen is an important biomarker for early nonculture diagnosis of invasive pulmonary aspergillosis in immunocompromised patients (8–10, 13). False-positive GM test results have been reported in patient serum or bronchoalveolar lavage (BAL) fluid in association with antibiotics, infant feeding, and some enteric bacteria (1, 2, 4, 12, 23). Plasma-Lyte infusion more recently has been reported to cause false-positive antigenemia (7, 15, 21).

Plasma-Lyte is a sterile, nonpyrogenic electrolyte solution containing sodium chloride, potassium chloride, magnesium chloride, sodium acetate trihydrate, and bicarbonate ions with or without sodium gluconate. It is used for fluid resuscitation, hydration in seriously ill patients (6, 11, 17, 20), and extended storage of platelets or peripheral blood stem cell products (3, 16, 19).

Hage et al. first described Plasma-Lyte as a cause of false-positive results for *Aspergillus* GM in BAL fluid (7). Surmont and Stockman then reported that giving a patient gluconate-containing Plasma-Lyte was the cause of false-positive serum GM (21). These two reports hypothesized that GM generated from *Aspergillus niger* during the industrial fermentation process of sodium gluconate was present in Plasma-Lyte solution, resulting in false-positive reactions. Racil and colleges subsequently reported a study where healthy volunteers receiving Plasma-Lyte demonstrated false-positive circulating GM lasting as long as 24 h (15). Whether sodium gluconate is the cause of false-positive GM reactions is not known.

We therefore conducted a series of experiments to test the hypothesis that the mechanism of false-positive GM reactions in Plasma-Lyte was the presence of sodium gluconate. Rabbits weighing 2.6 to 3.3 kg (Covance Research Products, Inc., Denver, PA) and monitored under humane care (14) received intravenous infusions of different formulations and lots of gluconate-containing Plasma-Lyte A or Plasma-Lyte 148 and a solution without gluconate Plasma-Lyte 56 via a Silastic tunneled central catheter (22). GM concentrations and GM indices (GMIs) were determined by using GM sandwich enzyme-linked immunosorbent assay (Platelia *Aspergillus* EIA; Bio-Rad, Marnes-la-Coquette, France). Serial serum GM level measurements in rabbits were analyzed using the Mann-Whitney or Kruskal-Wallis test. A two-tailed P value of <0.05 was considered to be statistically significant.

In order to understand the kinetics of circulating Plasma-Lyte-associated GM antigen, we gave rabbits intravenous infusions of single and multiple doses. A single 30-ml/kg dose of Plasma-Lyte solution was infused over 1 h. The multiple-dose study consisted of 20-ml/kg Plasma-Lyte infusions (1 ml/min) daily for 7 days, following by a 30-ml/kg bolus infusion on day 8. Serum specimens were collected at 0.17, 0.25, 0.5, 1, 2, 3, 4, 12, 14, 16, 24, 30, 36, and 48 h postinfusion in the single- and multiple-dose studies following the last infusion on day 8.

Assays were also conducted *in vitro* for the presence of GM antigen in different formulations and lots of gluconate-containing Plasma-Lyte solution and Plasma-Lyte without gluconate. Among the six lots of Plasma-Lyte A with sodium gluconate (502 mg/100 ml), the GMIs ranged from 3.28 ± 0.09 to 7.26 ± 0.01. Sodium gluconate-containing Plasma-Lyte 148 (502 mg/100 ml) *in vitro* contained the highest concentrations of GM antigen (GMI, 8.17 ± 0.15). In comparison, the GMIs of solutions without sodium gluconate (Plasma-Lyte 56) did not cause positive GM results (GMI, 0.16 ± 0.01; $P < 0.001$). Three to six GMI tests were performed on each batch of Plasma-Lyte solution.

We then compared serum GMI kinetics following infusions of Plasma-Lyte with and without sodium gluconate. The expression of circulating GM antigen in rabbits following an intravenous 30-ml/kg infusion of gluconate-containing Plasma-Lyte A ($n = 4$) or 148 ($n = 4$) solution with an *in vitro* GMI of 7.26 ± 0.01 or 8.17 ± 0.15 over 1 h is presented in Fig. 1A as a combined concentration-time curve. Following gluconate-

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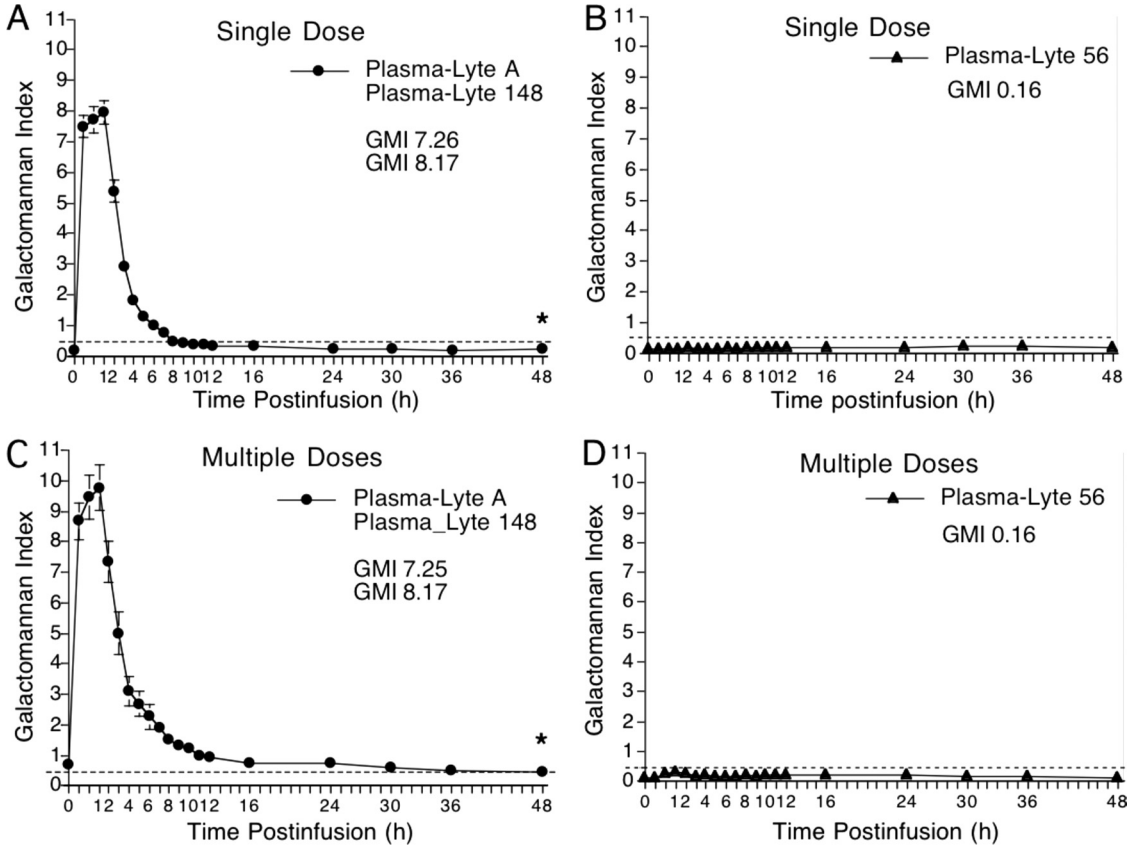

FIG. 1. Expression of GM antigen following the intravenous infusion of different formulations and lots of Plasma-Lyte solutions. (A) Combined concentration-time curve of single 30-ml/kg infusions each of gluconate-containing Plasma-Lyte A (lot C738476; GMI, 7.26 ± 0.01) and gluconate-containing Plasma-Lyte 148 (lot C730549; GMI, 8.17 ± 0.15) given over 1 h. (B) Single-dose 30-ml/kg infusion of a solution of Plasma-Lyte 56 (GMI, 0.16 ± 0.01) without gluconate administered over 1 h. (C) Combined concentration-time curve after the administration of multiple-dose gluconate-containing Plasma-Lyte A (lot C738476) and Plasma-Lyte 148 (lot C730549) infusions given over 1 h performed on day 8. (D) Multiple infusions of a solution of Plasma-Lyte 56 without gluconate given over 1 h performed on day 8. Values are presented as means ± stand errors of the means. *, $P < 0.0001$ (Kruskal-Wallis test).

containing Plasma-Lyte infusion, the serum GMI underwent significant changes over time ($P < 0.0001$, Kruskal-Wallis test). In comparison, there were no significant changes in serum GM in rabbits receiving a solution without gluconate Plasma-Lyte 56 ($n = 4$) (Fig. 1B).

We then studied serum GMI kinetics following repeated administration of Plasma-Lyte with or without gluconate over 7 days. Gluconate-containing Plasma-Lyte resulted in an accumulation of circulating GM to a peak GMI of 9.74 ± 0.75 after 1 h (Fig. 1C). The serum GMI remained positive for more than 36 h (0.54 ± 0.03) following the administration of Plasma-Lyte solution containing sodium gluconate, while there were no changes in the kinetics of GM antigen in serum following multiple administrations of a Plasma-Lyte 56 solution without sodium gluconate (GMI, 0.16 ± 0.01; Fig. 1D). Serum GM levels remained very low (GMI, 0.12 ± 0.03).

We further sought to demonstrate whether there is a direct relationship between serum GMI and infused volume and the *in vitro* GMI of Plasma-Lyte solution. Following a 10-ml/kg Plasma-Lyte A infusion (GMI, 4.18 ± 0.01) ($n = 4$), the serum GMI increased to 2.47 ± 0.32 in 10 min (Fig. 2A). With an increase in the volume of Plasma-Lyte A to 30 ml/kg (GMI, 4.18 ± 0.01), the serum GMI significantly increased over time to 3.68 ± 0.38 ($n = 6$) (Fig. 2B; $P < 0.0001$, Kruskal-Wallis test).

Formulations of sodium gluconate-containing Plasma-Lyte solutions produced distinctively high levels of GM antigen *in vitro* and *in vivo*. In comparison, Plasma-Lyte formulations without sodium gluconate did not cause false-positive results *in vitro* or false-positive antigenemia. Thus, there was a direct relationship between the GM concentrations in gluconate-containing Plasma-Lyte and GMI levels in serum. The persistence of antigenemia in animals for ≥24 h after infusion reported here was similar to that reported in human volunteers by Racil et al. (15).

To our knowledge, this is the first study to demonstrate that sodium gluconate in Plasma-Lyte is directly related to the cause of false-positive antigenemia. The present study demonstrates that Plasma-Lyte solutions that contain gluconate also contain high levels of GM antigen and cause false-positive circulating GM results after parenteral administration. Low-molecular-weight organic acids, such as gluconate, are produced by a fermentation process involving *A. niger* and *A. terreus* (5, 18). Calcium gluconate is also used in medical settings and may potentially be a cause of false-positive antigenemia. GM is released into the fermentation solution and is

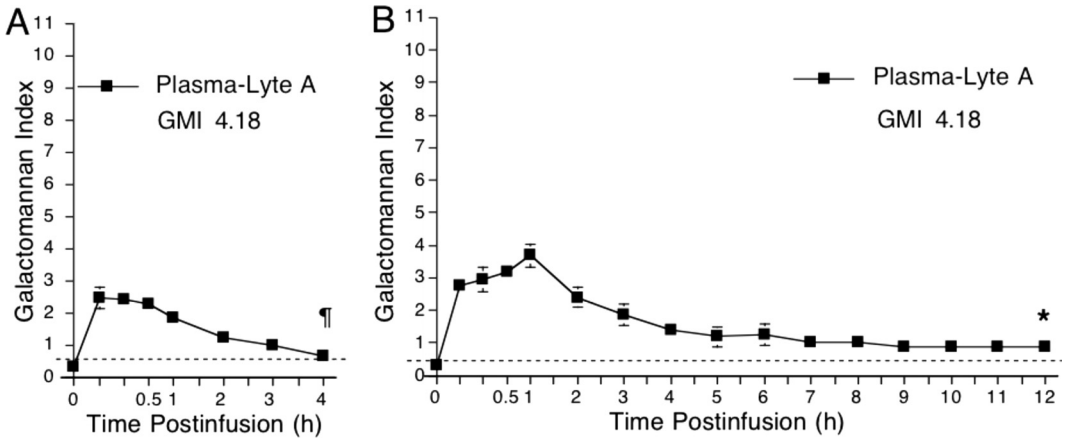

FIG. 2. Expression of GM antigen following intravenous infusion of gluconate-containing Plasma-Lyte A (lot C689893; GMI 4.18 ± 0.01). (A) A 10-ml/kg infusion administered over 10 min. (B) A 30-ml/kg infusion administered over 1 h. Values are presented as means ± stand errors of the means. ¶, $P = 0.0002$ (Mann-Whitney test); *, $P < 0.0001$ (Kruskal-Wallis test).

likely carried through the fractionation process for medical grade gluconate. Although Plasma-Lyte is sterile, GM still persists in the gluconate-containing solution.

In summary, this study demonstrates the role of gluconate in the GM antigenicity of Plasma-Lyte solution. Intravenously administered sodium gluconate-containing, but not gluconate-free, Plasma-Lyte produces a concentration- and rate-dependent serum GM signal. Awareness of this source of false-positive GM antigen may allow improved interpretation by clinical microbiology laboratories and improved patient care.

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